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(54) Title: PROCESS FOR CONTROLLING LEPIDOPTERAN PESTS (57) Abstract The subject disclosure concerns <i>Bacillus thuringiensis</i> strains which can be used to control lepidopteran pests. The strains were previously known to control coleopteran pests. The discovery of lepidopteran activity was totally unexpected. These <i>B.t.</i> strains can be formulated using standard lepidopteran formulation procedures. Means of administration are also standard. The genes encoding lepidopteran-active toxins can be isolated from the <i>B.t.</i> isolates and used to transform other microbes or plants for use to control lepidopteran pests.		

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DESCRIPTIONPROCESS FOR CONTROLLING LEPIDOPTERAN PESTSCross-Reference to Related Applications

This application is a continuation-in-part of co-pending U.S. application Serial No. 07/758,020, filed September 12, 1991, which is a continuation-in-part of co-pending U.S. application Serial No. 07/642,112, filed January 16, 1991, now abandoned. Serial No. 07/758,020 is also a continuation-in-part of U.S. application Serial No. 07/658,935, filed February 21, 1991.

Background of the Invention

The soil microbe *Bacillus thuringiensis* (*B.t.*) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These often appear microscopically as distinctively shaped crystals. The proteins are highly toxic to pests and specific in their activity. The toxin genes have been isolated and sequenced, and recombinant DNA-based *B.t.* products produced and approved. In addition, with the use of genetic engineering techniques, new approaches for delivering *B.t.* endotoxins to agricultural environments are under development, including the use of plants genetically engineered with endotoxin genes for insect resistance and the use of stabilized intact microbial cells as *B.t.* endotoxin delivery vehicles (Gaertner, F.H., L. Kim [1988] *TIBTECH* 6:S4-S7). Thus, isolated *B.t.* endotoxin genes are becoming commercially valuable.

Bacillus thuringiensis produces a proteinaceous paraspore or crystal which is toxic upon ingestion by a susceptible insect host. Over the past 30 years, commercial use of *B.t.* pesticides has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of *B. thuringiensis* subsp. *kurstaki* have been used for many years as commercial insecticides for lepidopteran pests. For example, *B. thuringiensis* var. *kurstaki* HD-1 produces a crystal called a delta endotoxin which is toxic to the larvae of a number of lepidopteran insects.

In recent years, however, investigators have discovered *B.t.* pesticides with specificities for a much broader range of pests. For example, other species of *B.t.*, namely *israelensis* and *san diego* (a.k.a. *B.t. tenebrionis*, a.k.a. M-7), have been used commercially to control insects of the orders Diptera and Coleoptera, respectively (Gaertner, F.H. [1989] "Cellular Delivery Systems for Insecticidal Proteins: Living and Non-Living Microorganisms," in *Controlled Delivery of Crop Protection Agents*, R.M. Wilkins, ed., Taylor and Francis, New York and London, 1990, pp. 245-255). See also Couch, T.L. (1980) "Mosquito Pathogenicity of *Bacillus thuringiensis* var. *israelensis*," *Developments in Industrial Microbiology* 22:61-76; Beegle, C.C., (1978) "Use of Entomogenous Bacteria in Agroecosystems," *Developments in Industrial Microbiology* 20:97-104.

Krieg, A., A.M. Huger, G.A. Langenbruch, W. Schnetter (1983) *Z. ang. Ent.* 96:500-508, describe a *B.t.* isolate named *Bacillus thuringiensis* var. *tenebrionis*, which is reportedly active against two beetles in the order Coleoptera. These are the Colorado potato beetle, *Leptinotarsa decemlineata*, and *Agelastica alni*.

5 Recently, many new subspecies of *B.t.* have been identified, and many genes responsible for active δ -endotoxin proteins have been isolated (Höfte, H., H.R. Whiteley [1989] *Microbiological Reviews* 52(2):242-255). Höfte and Whiteley classified 42 *B.t.* crystal protein genes into 14 distinct genes, grouped into 4 major classes based on amino-acid sequence and host range. The classes were CryI (Lepidoptera-specific), CryII (Lepidoptera- and Diptera-specific), CryIII (Coleoptera-specific), and CryIV (Diptera-specific). The discovery of strains specifically toxic to
10 protozoan pathogens, animal-parasitic liver flukes (Trematoda), or mites (Acar) has broadened the potential *B.t.* product spectrum even further. With activities against unique targets, these novel strains retain their very high biological specificity; nontarget organisms remain unaffected. The availability of a large number of diverse *B.t.* toxins may also enable farmers to adopt product-use strategies that minimize the risk that *B.t.*-resistant pests will arise.

15 The cloning and expression of a *B.t.* crystal protein gene in *Escherichia coli* has been described in the published literature (Schnepf, H.E., H.R. Whiteley [1981] *Proc. Natl. Acad. Sci. USA* 78:2893-2897). U.S. Patent 4,448,885 and U.S. Patent 4,467,036 both disclose the expression of *B.t.* crystal protein in *E. coli*. U.S. Patent 4,853,331 discloses *B. thuringiensis* strain *san diego* (a.k.a. *B.t. tenebrionis*, a.k.a. M-7) which can be used to control coleopteran pests in various
20 environments. U.S. Patent No. 4,849,217 discloses *Bacillus thuringiensis* isolates active against the alfalfa weevil. One of the isolates disclosed is *B. thuringiensis* PS86A1 (NRRL B-18400).

Brief Summary of the Invention

25 The subject invention concerns a novel process for controlling lepidopteran pests. This process results from the unexpected discovery that certain coleopteran-active *B.t.* isolates also have activity against lepidopteran pests, e.g., the diamondback moth (*Plutella xylostella*). This discovery was particularly surprising because known coleopteran-active isolates such as *Bacillus thuringiensis* var. *tenebrionis* (Krieg *et al.*, *supra*) (hereinafter referred to as M-7) are not toxic to Lepidoptera.

30 More specifically, the subject invention process uses *B.t.* microbes, or variants thereof, and/or their toxins, to control lepidopteran pests. Specific *B.t.* microbes useful according to the invention are *B.t.* PS86A1, *B.t.* PSS0C, and *B.t.* PS43F. Further, the subject invention also includes the use of variants of the *B.t.* isolates of the invention which have substantially the same lepidopteran-active properties as the specifically exemplified *B.t.* isolates. Procedures for making
35 mutants are well known in the microbiological art. Ultraviolet light and nitrosoguanidine are used extensively toward this end.

The subject invention also includes the use of genes from the *B.t.* isolates of the invention which genes encode the lepidopteran-active toxins.

Still further, the invention also includes the treatment of substantially intact *B.t.* cells, and recombinant cells containing the genes of the invention, to prolong the lepidopteran activity when the substantially intact cells are applied to the environment of a target pest. Such treatment can be by chemical or physical means, or a combination of chemical and physical means, so long as the technique does not deleteriously affect the properties of the pesticide, nor diminish the cellular capability in protecting the pesticide. The treated cell acts as a protective coating for the pesticidal toxin. The toxin becomes available to act as such upon ingestion by a target insect.

Finally, the subject invention further concerns plants which have been transformed with genes encoding lepidopteran-active toxins.

Brief Description of the Sequences

SEQ ID NO. 1 is the nucleotide sequence (open reading frame only) of the gene designated 50C.

SEQ ID NO. 2 is the predicted amino acid sequence of the toxin 50C.

SEQ ID NO. 3 is the composite nucleotide and amino acid sequences of the gene designated 43F.

SEQ ID NO. 4 is the predicted amino acid sequence of the toxin 43F.

SEQ ID NO. 5 is the nucleotide sequence (open reading frame only) of the gene designated 86A1.

SEQ ID NO. 6 is the predicted amino acid sequence of the toxin 86A1.

SEQ ID NO. 7 is an oligonucleotide probe designated 86A1-A.

Detailed Disclosure of the Invention

The *Bacillus thuringiensis* isolates useful according to the subject invention have the following characteristics in their biologically pure form:

Characteristics of *B.t.* PS50C

Colony morphology--Large colony, dull surface, typical *B.t.*

Vegetative cell morphology--typical *B.t.*

Culture methods--typical for *B.t.*

Flagellar serotyping--PS50C belongs to serotype 18, kumamotoensis.

Crystal morphology--a sphere.

RFLP analysis--Southern hybridization of total DNA distinguishes *B.t.* PS50C from

B.t.s.d. and other *B.t.* isolates.

Alkali-soluble proteins--SDS polyacrylamide gel electrophoresis (SDS-PAGE) shows a 130 kDa doublet protein.

The characteristics of *B.t.* PS86A1 with regard to colony morphology, vegetative cell morphology and culture methods are as given above for *B.t.* PS50C. However, these isolates differ, as shown in Table 1, with respect to inclusions, serotype, and molecular weights of toxins.

B.t. PS43F is disclosed in U.S. Patent 4,996,155.

A comparison of the characteristics of the *B. thuringiensis* strains of the subject invention to the characteristics of the known *B.t.* strains *B. thuringiensis* var. *tenebrionis* (M-7) and *B. thuringiensis* var. *kurstaki* (HD-1) is shown in Table 1.

Table 1. Comparison of *B.t.* PS50C, *B.t.* PS86A1, *B.t.* PS43F, *B.t.* (M-7) and *B.t.* HD-1

	<i>B.t.</i> PS50C	<i>B.t.</i> PS86A1	<i>B.t.</i> PS43F	<i>B.t.</i> HD-1	M-7
Inclusions:	Sphere	Multiple attached	Flat, pointed, ellipse, plus small inclusions	Bipyramid	Flat square
Approximate molecular wt. of proteins by SDS-PAGE (kDa)	130,000 doublet	58,000 45,000	75,000 68,000 61,000	130,000 68,000	72,000 64,000
Serotype	kumamotoensis	wuhenensis	tolworthi	kurstaki	morrisoni

B.t. isolates useful according to the subject invention have been deposited. Also deposited are recombinant microbes comprising the *B.t.* genes of interest.

Culture	Accession Number	Deposit Date
<i>Bacillus thuringiensis</i> PS50C	NRRL B-18746	January 9, 1991
<i>E. coli</i> NM522(pMYC1638)	NRRL B-18751	January 11, 1991
<i>Bacillus thuringiensis</i> PS86A1	NRRL B-18400	August 16, 1988
<i>E. coli</i> NM522(pMYC2320)	NRRL B-18769	February 14, 1991
<i>Bacillus thuringiensis</i> PS43F	NRRL B-18298	February 2, 1988
<i>E. coli</i> XL1-Blue (pM1,98-4)	NRRL B-18291	January 15, 1988

The cultures are on deposit in the permanent collection of the Northern Research Laboratory, U.S. Department of Agriculture, Peoria, IL, USA.

The subject cultures have been deposited under conditions that assure that access to the cultures will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 USC 122. The deposits are available as required by foreign patent laws in countries wherein

counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposits will be stored and made available to the public in
5 accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the cultures. The depositor
10 acknowledges the duty to replace the deposits should the depository be unable to furnish a sample when requested, due to the condition of the deposit(s). All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

The lepidopteran toxin genes of the subject invention can be isolated by known
15 procedures and can be introduced into a wide variety of microbial hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. With suitable hosts, e.g., *Pseudomonas*, the microbes can be applied to the situs of lepidopteran insects where they will proliferate and be ingested by the insects. The result is a control of the unwanted insects. Alternatively, the microbe hosting the toxin gene can be treated
20 under conditions that prolong the activity of the toxin produced in the cell. The treated cell then can be applied to the environment of target pest(s). The resulting product retains the toxicity of the *B.t.* toxin.

Where the *B.t.* toxin gene is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is important that certain host microbes
25 be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably,
30 provide for improved protection of the pesticide from environmental degradation and inactivation.

A large number of microorganisms are known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*,
35 *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, e.g., genera *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*,

Rhodotorula, and *Aureobasidium*. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Acetobacter xylinum*, *Agrobacterium tumefaciens*, *Rhodopseudomonas spheroides*, *Xanthomonas campestris*, *Rhizobium* *melloti*, *Alcaligenes entrophus*, and *Azotobacter vinlandii*; and phytosphere yeast species such as *Rhodotorula rubra*, *R. glutinis*, *R. marina*, *R. aurantiaca*, *Cryptococcus albidus*, *C. diffluens*, *C. laurentii*, *Saccharomyces rosei*, *S. pretoriensis*, *S. cerevisiae*, *Sporobolomyces roseus*, *S. odoratus*, *Khuyveromyces veronae*, and *Aureobasidium pollulans*. Of particular interest are the pigmented microorganisms.

A wide variety of ways are available for introducing the *B.t.* gene expressing the toxin into the microorganism host under conditions which allow for stable maintenance and expression of the gene. One can provide for DNA constructs which include the transcriptional and translational regulatory signals for expression of the toxin gene, the toxin gene under their regulatory control and a DNA sequence homologous with a sequence in the host organism, whereby integration will occur, and/or a replication system which is functional in the host, whereby integration or stable maintenance will occur.

The transcriptional initiation signals will include a promoter and a transcriptional initiation start site. In some instances, it may be desirable to provide for regulative expression of the toxin, where expression of the toxin will only occur after release into the environment. This can be achieved with operators or a region binding to an activator or enhancers, which are capable of induction upon a change in the physical or chemical environment of the microorganisms. For example, a temperature sensitive regulatory region may be employed, where the organisms may be grown up in the laboratory without expression of a toxin, but upon release into the environment, expression begins. Other techniques may employ a specific nutrient medium in the laboratory, which inhibits the expression of the toxin, where the nutrient medium in the environment allows for expression of the toxin. For translational initiation, a ribosomal binding site and an initiation codon will be present.

Various manipulations may be employed for enhancing the expression of the messenger, particularly by using an active promoter, as well as by employing sequences, which enhance the stability of the messenger RNA. The initiation and translational termination region will involve stop codon(s), a terminator region, and optionally, a polyadenylation signal.

In the direction of transcription, namely in the 5' to 3' direction of the coding or sense sequence, the construct can involve the transcriptional regulatory region, if any, and the promoter, where the regulatory region may be either 5' or 3' of the promoter, the ribosomal binding site, the initiation codon, the structural gene having an open reading frame in phase with the initiation codon, the stop codon(s), the polyadenylation signal sequence, if any, and the terminator region. This sequence as a double strand may be used by itself for transformation of a microorganism host, but will usually be included with a DNA sequence involving a marker, where the second

DNA sequence may be joined to the toxin expression construct during introduction of the DNA into the host.

By a marker is intended a structural gene which provides for selection of those hosts which have been modified or transformed. The marker will normally provide for selective advantage, for example, providing for biocide resistance, e.g., resistance to antibiotics or heavy metals; complementation, so as to provide prototrophy to an auxotrophic host, or the like. Preferably, complementation is employed, so that the modified host may not only be selected, but may also be competitive in the field. One or more markers may be employed in the development of the constructs, as well as for modifying the host. The organisms may be further modified by providing for a competitive advantage against other wild-type microorganisms in the field. For example, genes expressing metal chelating agents, e.g., siderophores, may be introduced into the host along with the structural gene expressing the toxin. In this manner, the enhanced expression of a siderophore may provide for a competitive advantage for the toxin-producing host, so that it may effectively compete with the wild-type microorganisms and stably occupy a niche in the environment.

Where stable episomal maintenance or integration is desired, a plasmid will be employed which has a replication system which is functional in the host. The replication system may be derived from the chromosome, an episomal element normally present in the host or a different host, or a replication system from a virus which is stable in the host. A large number of plasmids are available, such as pBR322, pACYC184, RSF1010, pRO1614, and the like. See for example, Olson *et al.* (1982) *J. Bacteriol.* 150:6069, and Bagdasarian *et al.* (1981) *Gene* 16:237, and U.S. Patent Nos. 4,356,270, 4,362,817, and 4,371,625.

Where no functional replication system is present, the construct will also include a sequence of at least 50 basepairs (bp), preferably at least about 100 bp, and usually not more than about 1000 bp of a sequence homologous with a sequence in the host. In this way, the probability of legitimate recombination is enhanced, so that the gene will be integrated into the host and stably maintained by the host. Desirably, the toxin gene will be in close proximity to the gene providing for complementation as well as the gene providing for the competitive advantage. Therefore, in the event that a toxin gene is lost, the resulting organism will be likely to also lose the complementing gene and/or the gene providing for the competitive advantage, so that it will be unable to compete in the environment with the gene retaining the intact construct.

A large number of transcriptional regulatory regions are available from a wide variety of microorganism hosts, such as bacteria, bacteriophage, cyanobacteria, algae, fungi, and the like. Various transcriptional regulatory regions include the regions associated with the *trp* gene, *lac* gene, *gal* gene, the lambda left and right promoters, the *tac* promoter, and the naturally-occurring promoters associated with the toxin gene, where functional in the host. See for example, U.S. Patent Nos. 4,332,898, 4,342,832 and 4,356,270. The termination region may be the termination

region normally associated with the transcriptional initiation region or a different transcriptional initiation region, so long as the two regions are compatible and functional in the host.

The *B.t.* gene can be introduced between the transcriptional and translational initiation region and the transcriptional and translational termination region, so as to be under the regulatory control of the initiation region. This construct can be included in a plasmid, which could include at least one replication system, but may include more than one, where one replication system is employed for cloning during the development of the plasmid and the second replication system is necessary for functioning in the ultimate host. In addition, one or more markers may be present, which have been described previously. Where integration is desired, the plasmid will desirably include a sequence homologous with the host genome.

The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against unmodified organisms or transferring organisms, when present. The transformants then can be tested for pesticidal activity.

Suitable host cells, where the pesticide-containing cells will be treated to prolong the activity of the toxin in the cell when the then treated cell is applied to the environment of target pest(s), may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxin is unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and -positive, include Enterobacteriaceae, such as *Escherichia*, *Erwinia*, *Shigella*, *Salmonella*, and *Proteus*; Bacillaceae; Rhizobiaceae, such as *Rhizobium*; Spirillaceae, such as photobacterium, *Zymomonas*, *Serratia*, *Aeromonas*, *Vibrio*, *Desulfovibrio*, *Spirillum*; Lactobacillaceae; Pseudomonadaceae, such as *Pseudomonas* and *Acetobacter*; Azotobacteraceae and Nitrobacteraceae. Among eukaryotes are fungi, such as Phycomycetes and Ascomycetes, which includes yeast, such as *Saccharomyces* and *Schizosaccharomyces*; and Basidiomycetes yeast, such as *Rhodotorula*, *Aureobasidium*, *Sporobolomyces*, and the like.

The cell will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instances spores may be employed.

Treatment of the microbial cell, e.g., a microbe containing the *B.t.* lepidopteran toxin gene, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability in protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable

techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Boulin's fixative and Helly's fixative (See: Humason, Gretchen L. [1967] *Animal Tissue Techniques*, W.H. Freeman and Company); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like.

The cells generally will have enhanced structural stability which will enhance resistance to environmental conditions. Where the pesticide is in a proform, the method of inactivation should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of inactivation or killing retains at least a substantial portion of the bio-availability or bioactivity of the toxin.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the *B.t.* gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Host organisms of particular interest include yeast, such as *Rhodotorula* sp., *Aureobasidium* sp., *Saccharomyces* sp., and *Sporobolomyces* sp.; phylloplane organisms such as *Pseudomonas* sp., *Erwinia* sp. and *Flavobacterium* sp.; or such other organisms as *Escherichia*, *Lactobacillus* sp., *Bacillus* sp., and the like. Specific organisms include *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, *Bacillus thuringiensis*, *Escherichia coli*, *Bacillus subtilis*, and the like.

The cellular host containing the *B.t.* lepidopteran gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the *B.t.* gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

The *B.t.* cells may be formulated in a variety of ways. They may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker

adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, gels, suspensions, emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

5 The pesticidal concentration will vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The pesticide will be present in at least about 1% by weight and may be about 100% by weight. The dry formulations will have from about 1-95% by weight of the pesticide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally
10 have from about 10^2 to about 10^4 cells/mg. These formulations will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare.

The formulations can be applied to the environment of the lepidopteran pest(s), e.g., plants, soil or water, by spraying, dusting, sprinkling, or the like.

15 Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 — Culturing *Bacillus thuringiensis* Isolates

20 A subculture of a *B.t.* isolate of the invention can be used to inoculate the following medium, a peptone, glucose, salts medium.

	Bacto Peptone	7.50 g/l
	Glucose	1.00 g/l
	KH_2PO_4	3.40 g/l
	K_2HPO_4	4.35 g/l
25	Salt Solution	5.00 ml/l
	CaCl_2 Solution	5.00 ml/l

pH 7.2

Salts Solution (100 ml)

	MgSO ₄ ·7H ₂ O	2.46 g
	MnSO ₄ ·H ₂ O	0.04 g
	ZnSO ₄ ·7H ₂ O	0.28 g
5	FeSO ₄ ·7H ₂ O	0.40 g

CaCl₂ Solution (100 ml)

	CaCl ₂ ·2H ₂ O	3.66 g
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10 The salts solution and CaCl₂ solution are filter-sterilized and added to the autoclaved and cooked broth at the time of inoculation. Flasks are incubated at 30°C on a rotary shaker at 200 rpm for 64 hr.

The above procedure can be readily scaled up to large fermentors by procedures well known in the art.

15 The *B.t.* spores and crystals, obtained in the above fermentation, can be isolated by procedures well known in the art. A frequently-used procedure is to subject the harvested fermentation broth to separation techniques, e.g., centrifugation.

Example 2 -- Cloning of a Toxin Gene from *B.t.* Isolate PS50C

20 Total cellular DNA was prepared from *Bacillus thuringiensis* (*B.t.*) cells grown to an optical density, at 600 nm, of 1.0. The cells were recovered by centrifugation and protoplasts were prepared in TES buffer (30 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, pH = 8.0) containing 20% sucrose and 50 mg/ml lysozyme. The protoplasts were lysed by addition of SDS to a final concentration of 4%. The cellular material was precipitated overnight at 4°C in 100 mM (final concentration) neutral potassium chloride. The supernate was extracted twice with
25 phenol/chloroform (1:1). Nucleic acids were precipitated with ethanol and DNA was purified by isopycnic banding on cesium chloride-ethidium bromide gradients.

30 Total cellular DNA from *B.t.* subsp. *kumamotoensis* (*B.t.* *kum.*), isolate PS50C, was digested with *Hind*III and fractionated by electrophoresis on a 0.8% (w/v) agarose-TAE (50 mM Tris-HCl, 20 mM NaOAc, 2.5 mM EDTA, pH = 8.0) buffered gel. A Southern blot of the gel was hybridized with a [³²P]-radiolabeled oligonucleotide probe. Results showed that the hybridizing fragments of PS50C are approximately 12 kb and 1.7 kb in size.

35 A library was constructed from PS50C total cellular DNA partially digested with *Sau*3A and size fractionated by gel electrophoresis. The 9-23 kb region of the gel was excised and the DNA was electroeluted and then concentrated using an Elutip-dTM ion exchange column (Schleicher and Schuel, Keene, NH). The isolated *Sau*3A fragments were ligated into *Bam*HI-digested LambdaGEM-11TM (PROMEGA). The packaged phage were plated on *E. coli* KW251

cells (PROMEGA) at a high titer and screened using the radiolabeled oligonucleotide probe. Hybridizing plaques were purified and rescreened at a lower plaque density. Single isolated, purified plaques that hybridized with the probe were used to infect *E. coli* KW251 cells in liquid culture for preparation of phage for DNA isolation. DNA was isolated by standard procedures. Preparative amounts of DNA were digested with *Xho*I (to release the inserted DNA from lambda sequences) and separated by electrophoresis on a 0.6% agarose-TAE gel. The large fragments were purified by ion exchange chromatography as above and ligated to *Xho*I-digested, dephosphorylated pHTBlueII (an *E. coli*/*B. thuringiensis* shuttle vector comprised of pBluescript s/k [Stratagene] and the replication origin from a resident *B.t.* plasmid [D. Lereclus *et al.* [1989] *FEMS Microbiology Letters* 60:211-218]). The ligation mix was introduced by transformation into competent *E. coli* NM522 cells (ATCC 47000) and plated on LB agar containing ampicillin, isopropyl-(β)-D-thiogalactoside (IPTG) and 5-bromo-4-chloro-4-indolyl-(β)-D-galactoside (XGAL). White colonies, with putative restriction fragment insertions in the (β)-galactosidase gene of pHTBlueII, were subjected to standard rapid plasmid purification procedures. Plasmids were analyzed by *Xho*I digestion and agarose gel electrophoresis. The desired plasmid construct, pMYC1638, contains an approximately 12 kb *Xho*I insert. The nucleotide sequence (open reading frame only) is shown in SEQ ID NO. 1. The predicted amino acid sequence of the toxin is shown in SEQ ID NO. 2.

Plasmid pMYC1638 was introduced into an acrySTALLIFEROUS (Cry⁻) *B.t.* host (HD-1 cryB obtained from A. Aronson, Purdue University) by electroporation. Expression of an approximately 130 kDa protein was verified by SDS-PAGE.

Plasmid pMYC1638 containing the *B.t.* toxin gene, can be removed from the transformed host microbe by use of standard well-known procedures. For example, *E. coli* NM522[pMYC1638] NRRL B-18751 can be subjected to cleared lysate isopycnic density gradient procedures, and the like, to recover pMYC1638.

Example 3 - Cloning of Toxin Gene From *B.t.* Isolate PS43F and Transformation into *Pseudomonas*

Total cellular DNA was prepared by growing the cells of *B.t.* isolate PS43F and M-7 to a low optical density ($OD_{600} = 1.0$) and recovering the cells by centrifugation. The cells were protoplasted in a buffer containing 20% sucrose and 50 mg/ml lysozyme. The protoplasts were lysed by addition of SDS to a final concentration of 4%. The cellular material was precipitated overnight at 4°C in 100 mM neutral potassium chloride. The supernate was phenol/chloroform extracted twice and the DNA precipitated in 68% ethanol. The DNA was purified on a cesium chloride gradient. DNAs from strains 43F and M-7 (as a standard of reference) were digested with *Eco*RI and run out on a 0.8% agarose gel. The gel was Southern blotted and probed with the nick translated ORF *Xmn*I to *Pst*I fragment of the toxin encoding gene isolated from M-7 (this

will be subsequently referred to as Probe). The results showed 43F to hybridize to Probe at 7.5 kb which is different than the standard.

Preparative amounts of 43F DNA were digested with *EcoRI* and run out on a 0.8% agarose gel. The 7.5 kb region of the preparative gel was isolated and the DNA electroeluted and concentrated using an ELUTIPTM-d (Schleicher and Schuell, Keene, NH) ion exchange column. A sample was blotted and probed to verify the fragment was indeed isolated. The 7.5 kb *EcoRI* fragment was ligated to Lambda ZAPTM *EcoRI* arms. The packaged recombinant phage were plated out with *E. coli* strain BB4 (Stratagene Cloning Systems, La Jolla, CA) to give high plaque density.

The plaques were screened by standard procedures with Probe. The plaques that hybridized were purified and re-screened at a lower plaque density. The resulting phage were grown with M13 helper phage (Stratagene) and the recombinant BLUESCRIPTTM plasmid was automatically excised and packaged. The "phagemid" was re-infected in XL1-blue *E. coli* cells (Stratagene) as part of the automatic excision process. The infected XL1-blue cells were screened for ampicillin resistance and the resulting colonies were miniprep'd to find the desired plasmid pM1,98-4. The recombinant *E. coli* XL1-Blue (pM1,98-4) strain is called MR381.

The plasmid pM1,98-4 contained a 7.5 kb *EcoRI* insert. To verify that this insert was the one of interest, a Southern blot was performed and probed. The 7.5 kb band hybridized with Probe, confirming that the fragment had been cloned. Restriction endonuclease analysis of the 7.5 kb *EcoRI* fragment with the enzymes *HindIII*, *PstI*, *SpeI*, *BamHI* and *XbaI* was done to show that a gene different from M-7 had been cloned. The enzymes which cut inside the 7.5 kb *EcoRI* fragment were *HindIII* (twice) *SpeI* (twice) and *PstI* (once). The open reading frame (ORF) of the 43F gene cut once with *HindIII*, twice with *SpeI* and did not cut with *XbaI*, *EcoRI*, or *BamHI*. Sequence data showed an open reading frame of 1963 bp with at best 70% sequence similarity to the toxin encoding gene of M-7.

The cloned toxin gene from PS43F can be modified for expression in *P. fluorescens* in the following way:

(1) A plasmid containing the *Ptac*-promoted *cryIA(b)*-like toxin gene can be made using a 3-way ligation involving the *Ptac* promoter and toxin gene on a *BamHI*-*PstI* fragment of about 4500 bp from pM3,130-7 (from MR420, NRRL B-18332, disclosed in U.S. Patent No. 5,055,294), a *NorI*-*BamHI* fragment of about 5500 bp from pTJS260 (containing the tetracycline resistance genes, available from Dr. Donald Helinski, U.C. San Diego), and a *NorI*-*PstI* fragment of about 6100 bp from pTJS260 (containing the replication region). The assembled plasmid is recovered following transformation of *E. coli* and growth under tetracycline selection.

(2) A plasmid containing the *Ptac*-promoted 43F toxin gene can be made by ligating the toxin gene-containing *FspI*-*SspI* fragment of about 2200 bp from pM1,98-4 (from MR381(pM1,98-4), NRRL B-18291) into the *SmaI* site of the *E. coli* vector, pKK223-3 (Pharmacia). The *Ptac*-

promoted 43F toxin plasmid can be recovered following transformation of *E. coli*, growth under ampicillin selection, and screening for plasmids with inserts in the proper orientation for expression from the *tac* promoter by techniques well known in the art.

(3) The *Ptac*-promoted 43F toxin can be assembled into, for example, the pTJS260-derived vector in a three-way ligation using the 12.6 kb DNA fragment having *Bam*HI and filled-in *Nsi*I ends from the plasmid resulting from step 1 above, to the *Bam*HI-*Nsi*I *Ptac*-containing fragment of about 1.2 kb and the *Nsi*I-*Sca*I fragment of about 2.1 kb containing the 3' end of the 43F toxin gene and adjacent vector DNA from the plasmid resulting from step 2 above.

The resulting pTJS260-derived 43F toxin expression plasmid can be introduced into *Pseudomonas fluorescens* by electroporation.

The above cloning procedures were conducted using standard procedures unless otherwise noted.

The various methods employed in the preparation of the plasmids and transformation of host organisms are well known in the art. These procedures are described in Maniatis, T., E.F. Fritsch, J. Sambrook (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York. Thus, it is within the skill of those in the genetic engineering art to extract DNA from microbial cells, perform restriction enzyme digestions, electrophorese DNA fragments, tail and anneal plasmid and insert DNA, ligate DNA, transform cells, prepare plasmid DNA, electrophorese proteins, and sequence DNA.

The restriction enzymes disclosed herein can be purchased from Boehringer Mannheim, Indianapolis, IN, or New England BioLabs, Beverly, MA. The enzymes were used according to the instructions provided by the supplier.

Plasmid pM1,98-4 containing the *Bt* toxin gene, can be removed from the transformed host microbe by use of standard well-known procedures. For example, *E. coli* XL1-Blue (pM1,98-4) can be subjected to cleared lysate isopycnic density gradient procedures, and the like, to recover pM1,98-4.

Example 4 - Molecular Cloning of Gene Encoding a Toxin from *Bacillus thuringiensis* Strain PS86A1

Total cellular DNA was prepared from PS86A1 cells grown to an optical density, at 600 nm, of 1.0. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/ml lysozyme in 0.3 M sucrose, 25 mM Tris-Cl, pH 8.0, 25 mM EDTA). After incubation at 37°C for 1 hour, protoplasts were lysed by two cycles of freezing and thawing. Nine volumes of a solution of 0.1 M NaCl, 0.1% SDS, 0.1 M Tris-Cl were added to complete lysis. The cleared lysate was extracted twice with phenol:chloroform (1:1). Nucleic acids were precipitated with two volumes of ethanol and pelleted by centrifugation. The pellet was resuspended in 10 mM Tris-Cl, 1 mM EDTA (TE), pH 8.0, and RNase was added to a final concentration of 50 µg/ml. After

incubation at 37°C for 1 hour, the solution was extracted once each with phenol:chloroform (1:1) and TE-saturated chloroform. DNA was precipitated from the aqueous phase by the addition of one-tenth volume of 3 M NaOAc and two volumes of ethanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE.

5 Restriction fragment length polymorphism (RFLP) analyses were performed by standard hybridization of southern blots of PS86A1 DNA with a ³²P-labeled oligonucleotide probe designated as 86A1-A. The sequence of the 86A1-A probe was:

5' ATG ATT GAT TCT AAA ACA ACA TTA CCA AGA CAT TCT/A TTA ATT/A CAT
ACT/A ATT/A AA 3' (SEQ ID NO. 7)

10 The probe was mixed at four positions, as shown. Hybridizing bands included an approximately 3.6 kbp *Hind*III fragment and an approximately 9.3 kbp *Eco*RV fragment.

A gene library was constructed from PS86A1 DNA partially digested with *Sau*3A. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 6.6 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, and recovered by ethanol precipitation after purification on an Elutip-D ion exchange column. The *Sau*3A inserts were ligated into *Bam*HI-digested LambdaGem-11 (Promega, Madison, WI). Recombinant phage were packaged and plated on *E. coli* KW251 cells (Promega). Plaques were screened by hybridization with the radiolabeled 86A1-A oligonucleotide probe. Hybridizing phage were plaque-purified and used to infect liquid cultures of *E. coli* KW251 cells for isolation of phage DNA by standard procedures (Maniatis et al. [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). For subcloning, preparative amounts of DNA were digested with *Eco*RI and *Sal*I, and electrophoresed on an agarose gel. The approximately 2.9 kbp band containing the toxin gene was excised from the gel, electroeluted from the gel slice, and purified by ion exchange chromatography as above. The purified DNA insert was ligated into *Eco*RI + *Sal*I-digested pHTBlueII (an *E. coli*/B.t. shuttle vector comprised of pBlueScript S/K, Stratagene, San Diego, CA) and the replication origin from a resident B.t. plasmid (D. Lereclus et al. [1989] *FEMS Microbiol. Lett.* 60:211-218). The ligation mix was used to transform frozen, competent *E. coli* NM522 cells (ATCC 47000). Transformants were plated on LB agar (Maniatis et al., *supra*) containing ampicillin, isopropyl-(β)-D-thiogalactoside (IPTG), and 5-bromo-4-chloro-3-indolyl-(β)-D-galactoside (XGAL). Plasmids were purified from putative recombinants by alkaline lysis (Maniatis et al., *supra*) and analyzed by electrophoresis of *Eco*RI and *Sal*I digests on agarose gels. The desired plasmid construct, pMYC2320, contains the toxin gene of the invention. The DNA sequence of this gene is shown in SEQ ID NO. 5. The toxin expressed by this gene is shown in SEQ ID NO. 6.

35 Plasmid pMYC2320 was introduced into an acrySTALLIFEROUS (Cry⁻) B.t. host (B.t. HD-1 Cry B, A.L. Aronson, Purdue University, West Lafayette, IN) by electroporation. Expression of an approximately 58 kDa protein is verified by SDS-PAGE analysis.

Plasmid pMYC2320 containing the *B.t.* toxin gene, can be removed from the transformed host microbe by use of standard well-known procedures. For example, *E. coli* NMS22(pMYC2320) can be subjected to cleared lysate isopycnic density gradient procedures, and the like, to recover pMYC2320.

Example 5 - 43F Toxin Production by a Transformed *Pseudomonas fluorescens* Host

A transformed *Pseudomonas fluorescens* containing the PS43F gene was grown in the following medium using a 1% inoculum grown in LB medium with 30 µg/ml tetracycline:

Glycerol	65 g/L
Na citrate·2H ₂ O	7.14
HCT	20
Amberex 1003	20
NaNO ₃	5
(NH ₄) ₂ SO ₄	23

32°C at 300 rpm.

These were 72 hour fermentations with induction and supplementation taking place at 24 hours. They were induced with 2 mM IPTG and supplemented with the following:

Amisoy	20.0 g/L
MgSO ₄ ·7H ₂ O	0.4
K ₂ HPO ₄	1.6
KCl	1.6

The toxin concentration can be determined using laser densitometry (LKB) to quantify the approximately 70 kDa toxin protein found in the *P. fluorescens* host after Coomassie staining of polyacrylamide gels containing SDS.

Example 6 - Testing of *B.t.* Toxins Against the Diamondback Moth

(A) A spore crystal preparation of a *B.t.* clone comprising the PS86A1 gene was toxic to the lepidopteran pest, diamondback moth *Plutella xylostella*, in a 1.5% agar artificial diet assay. The *B.t.* clone was grown as disclosed in Example 1. Rates greater than 100 microgram protein/gram diet gave 100% control of this pest in 6 days.

(B) A spore crystal preparation of a *B.t.* clone comprising the PS50C gene was toxic to the lepidopteran pest, diamondback moth, in a 1.5% agar artificial diet assay. The *B.t.* clone was grown as disclosed in Example 1. Rates greater than 100 microgram protein/gram diet gave 100% control of this pest in 6 days.

(C) A *Pseudomonas fluorescens* clone comprising the PS43F gene was toxic to the lepidopteran pest, diamondback moth, in a 1.5% agar artificial diet assay. Rates greater than 40 microgram protein/gram diet gave 100% control of this pest in 6 days.

5 Example 7 – Further Testing of *B.t.* Toxins Against the Diamondback Moth

Toxins of the subject invention were produced by recombinant cells which had been transformed with genes according to the subject invention. The toxins produced by the recombinant cells were then tested for their activity against diamondback moths. The results of these experiments are shown in Table 2. These experiments were conducted as described in
10 Example 6.

Table 2				
Source strain	Cloned Toxin Gene	Host	Clone	Diamondback Moth LC ₅₀ (μg toxin/g diet)
15 PS86A1	86A1	<i>B. thuringiensis</i>	MR506	79
PS50C	50C	<i>B. thuringiensis</i>	MR505	19
PS43F	43F	<i>P. fluorescens</i>	MR816	11

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Example 8 – Insertion of Toxin Genes Into Plants

One aspect of the subject invention is the transformation of plants with genes encoding a lepidopteran toxin. The transformed plants are resistant to attack by lepidopterans.

Genes encoding lepidopteran-active toxins, as disclosed herein, can be inserted into plant
25 cells using a variety of techniques which are well known in the art. For example, a large number of cloning vectors comprising a replication system in *E. coli* and a marker that permits selection of the transformed cells are available for preparation for the insertion of foreign genes into higher plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence encoding the *B.t.* toxin can be inserted into the vector at a suitable
30 restriction site. The resulting plasmid is used for transformation into *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium, then harvested and lysed. The plasmid is recovered. Sequence analysis, restriction analysis, electrophoresis, and other biochemical-molecular biological methods are generally carried out as methods of analysis. After each manipulation, the DNA sequence used can be cleaved and joined to the next DNA sequence. Each plasmid sequence can
35 be cloned in the same or other plasmids. Depending on the method of inserting desired genes into the plant, other DNA sequences may be necessary. If, for example, the Ti or Ri plasmid is

used for the transformation of the plant cell, then at least the right border, but often the right and the left border of the Ti or Ri plasmid T-DNA, has to be joined as the flanking region of the genes to be inserted.

5 The use of T-DNA for the transformation of plant cells has been intensively researched and sufficiently described in EP 120 516; Hoekema (1985) In: *The Binary Plant Vector System*, Offset-drukkerij Kanters B.V., Alblasterdam, Chapter 5; Fraley *et al.*, *Crit. Rev. Plant Sci.* 4:1-46; and An *et al.* (1985) *EMBO J.* 4:277-287.

10 Once the inserted DNA has been integrated in the genome, it is relatively stable there and, as a rule, does not come out again. It normally contains a selection marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G 418, bleomycin, hygromycin, or chloramphenicol, *inter alia*. The individually employed marker should accordingly permit the selection of transformed cells rather than cells that do not contain the inserted DNA.

15 A large number of techniques are available for inserting DNA into a plant host cell. Those techniques include transformation with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation agent, fusion, injection, or electroporation as well as other possible methods. If agrobacteria are used for the transformation, the DNA to be inserted has to be cloned into special plasmids, namely either into an intermediate vector or into a binary vector. The intermediate vectors can be integrated into the Ti or Ri plasmid by homologous recombination owing to sequences that are homologous to sequences in the T-DNA. The Ti or Ri plasmid also comprises the *vir* region necessary for the transfer of the T-DNA. Intermediate vectors cannot replicate themselves in agrobacteria. The intermediate vector can be transferred into *Agrobacterium tumefaciens* by means of a helper plasmid (conjugation). Binary vectors can replicate themselves both in *E. coli* and in agrobacteria. They comprise a selection marker gene and a linker or polylinker which are framed by the right and left T-DNA border regions. They can be transformed directly into agrobacteria (Holsters *et al.* [1978] *Mol. Gen. Genet.* 163:181-187). The agrobacterium used as host cell is to comprise a plasmid carrying a *vir* region. The *vir* region is necessary for the transfer of the T-DNA into the plant cell. Additional T-DNA may be contained. The bacterium so transformed is used for the transformation of plant cells. Plant explants can advantageously be cultivated with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* for the transfer of the DNA into the plant cell. Whole plants can then be regenerated from the infected plant material (for example, pieces of leaf, segments of stalk, roots, but also protoplasts or suspension-cultivated cells) in a suitable medium, which may contain antibiotics or biocides for selection. The plants so obtained can then be tested for the presence of the inserted DNA. No special demands are made of the plasmids in the case of injection and electroporation. It is possible to use ordinary plasmids, such as, for example, pUC derivatives.

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The transformed cells grow inside the plants in the usual manner. They can form germ cells and transmit the transformed trait(s) to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

5

Example 9 – Cloning of Novel *B.t.* Genes Into Insect Viruses

A number of viruses are known to infect insects. These viruses include, for example, baculoviruses and entomopoxviruses. In one embodiment of the subject invention, lepidopteran-active genes, as described herein, can be placed with the genome of the insect virus, thus enhancing the pathogenicity of the virus. Methods for constructing insect viruses which comprise *B.t.* toxin genes are well known and readily practiced by those skilled in the art. These procedures are described, for example, in Merryweather *et al.* (Merryweather, A.T., U. Weyer, M.P.G. Harris, M. Hirst, T. Booth, R.D. Possee [1990] *J. Gen. Virol.* 71:1535-1544) and Martens *et al.* (Martens, J.W.M., G. Honee, D. Zuidema, J.W.M. van Lent, B. Visser, J.M. Vlak [1990] *Appl. Environmental Microbiol.* 56(9):2764-2770).

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It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Uyeda, Kendrick A.
Bradfish, Gregory A.
- (ii) TITLE OF INVENTION: Process for Controlling Lepidopteran Pests
- (iii) NUMBER OF SEQUENCES: 7
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- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
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 - (B) FILING DATE:
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 - (A) APPLICATION NUMBER: US 07/642,112
 - (B) FILING DATE: 16-JAN-1991
 - (C) CLASSIFICATION:
- (x) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 347 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) ORIGINAL SOURCE:
 - (A) ORGANISM: Bacillus thuringiensis
 - (B) STRAIN: kumamotoensis
 - (C) INDIVIDUAL ISOLATE: PS50C
- (vi) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli NM522(pMYC1638), NRRL B-18751

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGAGTCCAA ATAATCAAAA TGAATATGAA ATTATAGATG CGACACCTTC TACATCTGTA	60
TCCAGTGAAT CTAACAGATA CCCTTTTGGC AATGAGCCAA CAGATGCGTT ACAAAATATG	120
AATTATAAAG ATTATCTGAA AATGTCTGGG GGAGAGAATC CTGAATTATT TGGAAATCCG	180

GGAGCGTTTA	TTAGTTCATC	CACGATTCAA	ACTGGAATTG	GCATTGTTGG	TCGAATACTA	240
GGAGCTTTAG	GGGTTCCATT	TGCTAGTCAG	ATAGCTAGTT	TCTATAGTTT	CATTGTTGGT	300
CAATTATGGC	CGTCAAAGAG	CGTAGATATA	TGGGGAGAAA	TTATGGAACG	AGTGAAGAA	360
CTCGTTGATC	AAAAAATAGA	AAAATATGTA	AAAGATAAGG	CTCTTGCTGA	ATTTAAAGGG	420
CTAGGAAATG	CTTTGGATGT	ATATCAGCAG	TCACTTGAAG	ATTGGCTGGA	AAATCGCAAT	480
GATGCAAGAA	CTAGAAGTGT	TGTTTCTAAT	CAATTTATAG	CTTTAGATCT	TAACTTTGT	540
AGTTCAATTC	CATCTTTTGC	AGTATCCGGA	CACGAAGTAC	TATTATTAGC	AGTATATGCA	600
CAGGCTGTGA	ACCTACATTT	ATTGTTATTA	AGAGATGCTT	CTATTTTGG	AGAAGAGTGG	660
GGATTTACAC	CAGGTGAAAT	TTCTAGATTT	TATAATCGTC	AAGTGCAACT	TACCGCTGAA	720
TATTCAGACT	ATTGTGTAAA	GTGGTATAAA	ATCGGCTTAG	ATAAATTGAA	AGGTACCACT	780
TCTAAAAGTT	GGCTGAATTA	TCATCAGTTC	CGTAGAGAGA	TGACATTACT	GGTATTAGAT	840
TTGGTGGCGT	TATTTCCAAA	CTATGACACA	CATATGTATC	CAATCGAAAC	AACAGCTCAA	900
CTTACACGGG	ATGTGTATAC	AGATCCGATA	GCATTTAACA	TAGTGACAAG	TACTGGATTC	960
TGCAACCCCT	GGTCAACCCA	CAGTGGTATT	CTTTTATTATG	AAGTTGAAAA	CAACGTAATT	1020
CGTCCGCCAC	ACTTGTTTGA	TATACTCAGC	TCAGTAGAAA	TTAATACAAG	TAGAGGGGGT	1080
ATTACGTTAA	ATAATGATGC	ATATATAAAC	TACTGGTCAG	GACATACCCT	AAAATATCGT	1140
AGAACAGCTG	ATTCGACCGT	AACATACACA	GCTAATTACG	GTCGAATCAC	TTCAGAAAAG	1200
AATTCATTTG	CACCTGAGGA	TAGGGATATT	TTTGAATTA	ATTCAACTGT	GGCAAACCTA	1260
GCTAATTACT	ACCAAAAGGC	ATATGGTGTG	CCGGGATCTT	GGTTCCATAT	GGTAAAAGG	1320
GGAACCTCAT	CAACAACAGC	GTATTTATAT	TCAAAAACAC	ATACAGCTCT	CCAAGGGTGT	1380
ACACAGGTTT	ATGAATCAAG	TGATGAAATA	CCTCTAGATA	GAAGTGTACC	GGTAGCTGAA	1440
AGCTATAGTC	ATAGATTATC	TCATATTACC	TCCCATTCTT	TCTCTAAAAA	TGGGAGTGCA	1500
TACTATGGGA	GTTTCCCTGT	ATTTGTTTGG	ACACATACTA	GTGCGGATTT	AAATAATACA	1560
ATATATTTCAG	ATAAAATCAC	TCAAATTCCA	GCGGTAAAGG	GAGACATGTT	ATATCTAGGG	1620
GGTTCGGTAG	TACAGGGTCC	TGGATTTACA	GGAGGAGATA	TATTA AAAAG	AACCAATCCT	1680
AGCATATTAG	GGACCTTTGC	GGTTACAGTA	AATGGGTGCT	TATCACAAG	ATATCGTGTA	1740
AGAATTCGCT	ATGCCTCTAC	AACAGATTTT	GAATTTACTC	TATACCTTGG	CGACACAATA	1800
GAAAAAATA	GATTTAACAA	AACATATGGAT	AATGGGGCAT	CTTTAACGTA	TGAAACATTT	1860
AAATTCGCA	GTTTCATTAC	TGATTTCCAA	TTCAGAGAAA	CACAAGATAA	AATACTCCTA	1920
TCCATGGGTG	ATTTTAGCTC	CGGTCAAGAA	GTTTATATAG	ACCGAATCGA	ATTCATCCCA	1980
GTAGATGAGA	CATATGAGGC	GGAACAAGAT	TTAGAAGCGG	CGAAGAAAGC	AGTGAATGCC	2040
TTGTTTACGA	ATACAAAAGA	TGGCTTACGA	CCAGGTGTAA	CGGATTATGA	AGTAAATCAA	2100
GCGGCAAACT	TAGTGGAATG	CCTATCGGAT	GATTTATATC	CAAATGAAAA	ACGATTGTTA	2160
TTTGATGCGG	TGAGAGAGGC	AAAACGCCTC	AGTGGGGCAC	GTAACCTTACT	ACAAGATCCA	2220
GATTTCCAAG	AGATAAACGG	AGAAAATGGA	TGGGCGGCAA	GTACGGGAAT	TGAGATTGTA	2280
GAAGGGGATG	CTGTATTTAA	AGGACGTTAT	CTACGCCTAC	CAGGTGCACG	AGAAATTGAT	2340
ACGGAACCGT	ATCCAACGTA	TCTGTATCAA	AAAGTAGAGG	AAGGTGTATT	AAAACCATAC	2400
ACAAGATATA	GACTGAGAGG	GTTTGTGGGA	AGTAGTCAAG	GATTAGAAAT	TTATACGATA	2460
CGTCACCAA	CGAATCGAAT	TGTAAAGAAT	GTACCAGATG	ATTTATTGCC	AGATGTATCT	2520
CCTGTAAACT	CTGATGGCAG	TATCAATCGA	TGCAGCGAAC	AAAAGTATGT	GAATAGCCGT	2580
TTAGAAGGAG	AAAACCGTTC	TGGTGATGCA	CATGAGTTCT	CGCTCCCTAT	CGATATAGGA	2640
GAGCTGGATT	ACAATGAAAA	TGCAGGAATA	TGGGTTGGAT	TTAAGATTAC	GGACCCAGAG	2700

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GGATACGCAA CACTTGGAAA TCTTGAATTA GTCGAAGAGG GACCTTTGTC AGGAGACGCA	2760
TTAGAGCGCT TGCAAAGAGA AGAACAACAG TGAAGATTTC AAATGACAAG AAGACGTGAA	2820
GAGACAGATA GAAGATACAT GGCATCGAAA CAAGCGGTAG ATCGTTTATA TGCCGATTAT	2880
CAGGATCAAC AACTGAATCC TGATGTAGAG ATTACAGATC TTACTGCGGC TCAAGATCTG	2940
ATACAGTCCA TTCCTTACGT ATATAACGAA ATGTTCCAG AAATACCAGG GATGAATAT	3000
ACGAAGTTTA CAGAATTAA AGATCGACTC CAACAAGCGT GGAATTGTGTA TGATCAGCGA	3060
AATGCCATAC CAAATGGTGA TTTTCGAAT GGGTTAAGTA ATTGGAATGC AACGCTGGC	3120
GTAGAAGTAC AACAAATCAA TCATACATCT GTCCTTGTGA TTCCAAACTG GGATGAACAA	3180
GTTCACAAC AGTTTACAGT TCAACCGAAT CAAAGATATG TATTACGAGT TACTGCAAGA	3240
AAAGAAGGGG TAGGAAATGG ATATGTAAGT ATTCTGTATG GTGGAAATCA ATCAGAAACG	3300
CTTACTTTTA GTGCAAGCGA TTATGATACA AATGGTGTGT ATAATGACCA AACCGGCTAT	3360
ATCACAAAA CAGTGACATT CATCCCGTAT ACAGATCAA TGTGGATTGA AATAAGTGAA	3420
ACAGAAGGTA CGTCTATAT AGAAAGTGTA GAATTGATTG TAGACGTAGA G	3471

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1157 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Bacillus thuringiensis*
- (B) STRAIN: kumamotoensis
- (C) INDIVIDUAL ISOLATE: PS50C

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli NM522(pMYC1638), NRRL B-18751

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ser	Pro	Asn	Asn	Gln	Asn	Glu	Tyr	Glu	Ile	Ile	Asp	Ala	Thr	Pro
1				5					10					15	
Ser	Thr	Ser	Val	Ser	Ser	Asp	Ser	Asn	Arg	Tyr	Pro	Phe	Ala	Asn	Glu
			20					25					30		
Pro	Thr	Asp	Ala	Leu	Gln	Asn	Met	Asn	Tyr	Lys	Asp	Tyr	Leu	Lys	Met
		35					40					45			
Ser	Gly	Gly	Glu	Asn	Pro	Glu	Leu	Phe	Gly	Asn	Pro	Glu	Thr	Phe	Ile
	50					55					60				
Ser	Ser	Ser	Thr	Ile	Gln	Thr	Gly	Ile	Gly	Ile	Val	Gly	Arg	Ile	Leu
	65				70				75					80	
Gly	Ala	Leu	Gly	Val	Pro	Phe	Ala	Ser	Gln	Ile	Ala	Ser	Phe	Tyr	Ser
			85					90						95	
Phe	Ile	Val	Gly	Gln	Leu	Trp	Pro	Ser	Lys	Ser	Val	Asp	Ile	Trp	Gly
		100					105						110		
Glu	Ile	Met	Glu	Arg	Val	Glu	Glu	Leu	Val	Asp	Gln	Lys	Ile	Glu	Lys
		115				120						125			
Tyr	Val	Lys	Asp	Lys	Ala	Leu	Ala	Glu	Leu	Lys	Gly	Leu	Gly	Asn	Ala
	130				135					140					
Leu	Asp	Val	Tyr	Gln	Gln	Ser	Leu	Glu	Asp	Trp	Leu	Glu	Asn	Arg	Asn
	145				150				155					160	
Asp	Ala	Arg	Thr	Arg	Ser	Val	Val	Ser	Asn	Gln	Ph	Ile	Ala	Leu	Asp
				165				170						175	

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Leu Asn Phe Val Ser Ser Ile Pr Ser Phe Ala Val Ser Gly His Glu
 180 185 190
 Val Leu Leu Leu Ala Val Tyr Ala Gln Ala Val Asn Leu His Leu Leu
 195 200 205
 Leu Leu Arg Asp Ala Ser Ile Phe Gly Glu Glu Trp Gly Phe Thr Pro
 210 215 220
 Gly Glu Ile Ser Arg Phe Tyr Asn Arg Gln Val Gln Leu Thr Ala Glu
 225 230 235 240
 Tyr Ser Asp Tyr Cys Val Lys Trp Tyr Lys Ile Gly Leu Asp Lys Leu
 245 250 255
 Lys Gly Thr Thr Ser Lys Ser Trp Leu Asn Tyr His Gln Phe Arg Arg
 260 265 270
 Glu Met Thr Leu Leu Val Leu Asp Leu Val Ala Leu Phe Pro Asn Tyr
 275 280 285
 Asp Thr His Met Tyr Pro Ile Glu Thr Thr Ala Gln Leu Thr Arg Asp
 290 295 300
 Val Tyr Thr Asp Pro Ile Ala Phe Asn Ile Val Thr Ser Thr Gly Phe
 305 310 315 320
 Cys Asn Pro Trp Ser Thr His Ser Gly Ile Leu Phe Tyr Glu Val Glu
 325 330 335
 Asn Asn Val Ile Arg Pro Pro His Leu Phe Asp Ile Leu Ser Ser Val
 340 345 350
 Glu Ile Asn Thr Ser Arg Gly Gly Ile Thr Leu Asn Asn Asp Ala Tyr
 355 360 365
 Ile Asn Tyr Trp Ser Gly His Thr Leu Lys Tyr Arg Arg Thr Ala Asp
 370 375 380
 Ser Thr Val Thr Tyr Thr Ala Asn Tyr Gly Arg Ile Thr Ser Glu Lys
 385 390 395 400
 Asn Ser Phe Ala Leu Glu Asp Arg Asp Ile Phe Glu Ile Asn Ser Thr
 405 410 415
 Val Ala Asn Leu Ala Asn Tyr Tyr Gln Lys Ala Tyr Gly Val Pro Gly
 420 425 430
 Ser Trp Phe His Met Val Lys Arg Gly Thr Ser Ser Thr Thr Ala Tyr
 435 440 445
 Leu Tyr Ser Lys Thr His Thr Ala Leu Gln Gly Cys Thr Gln Val Tyr
 450 455 460
 Glu Ser Ser Asp Glu Ile Pro Leu Asp Arg Thr Val Pro Val Ala Glu
 465 470 475 480
 Ser Tyr Ser His Arg Leu Ser His Ile Thr Ser His Ser Phe Ser Lys
 485 490 495
 Asn Gly Ser Ala Tyr Tyr Gly Ser Phe Pro Val Phe Val Trp Thr His
 500 505 510
 Thr Ser Ala Asp Leu Asn Asn Thr Ile Tyr Ser Asp Lys Ile Thr Gln
 515 520 525
 Ile Pro Ala Val Lys Gly Asp Met Leu Tyr Leu Gly Gly Ser Val Val
 530 535 540
 Gln Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Lys Arg Thr Asn Pro
 545 550 555 560
 Ser Ile Leu Gly Thr Phe Ala Val Thr Val Asn Gly Ser Leu Ser Gln
 565 570 575
 Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asp Phe Glu Phe
 580 585 590
 Thr Leu Tyr Leu Gly Asp Thr Ile Glu Lys Asn Arg Phe Asn Lys Thr
 595 600 605
 Met Asp Asn Gly Ala Ser Leu Thr Tyr Glu Thr Phe Lys Phe Ala Ser
 610 615 620

Phe Ile Thr Asp Phe Gln Phe Arg Glu Thr Gln Asp Lys Ile Leu Leu
 625 630 635 640
 Ser Met Gly Asp Phe Ser Ser Gly Gln Glu Val Tyr Ile Asp Arg Ile
 645 650 655
 Glu Phe Ile Pro Val Asp Glu Thr Tyr Glu Ala Glu Gln Asp Leu Glu
 660 665 670
 Ala Ala Lys Lys Ala Val Asn Ala Leu Phe Thr Asn Thr Lys Asp Gly
 675 680 685
 Leu Arg Pro Gly Val Thr Asp Tyr Glu Val Asn Gln Ala Ala Asn Leu
 690 695 700
 Val Glu Cys Leu Ser Asp Asp Leu Tyr Pro Asn Glu Lys Arg Leu Leu
 705 710 715 720
 Phe Asp Ala Val Arg Glu Ala Lys Arg Leu Ser Gly Ala Arg Asn Leu
 725 730 735
 Leu Gln Asp Pro Asp Phe Gln Glu Ile Asn Gly Glu Asn Gly Trp Ala
 740 745 750
 Ala Ser Thr Gly Ile Glu Ile Val Glu Gly Asp Ala Val Phe Lys Gly
 755 760 765
 Arg Tyr Leu Arg Leu Pro Gly Ala Arg Glu Ile Asp Thr Glu Thr Tyr
 770 775 780
 Pro Thr Tyr Leu Tyr Gln Lys Val Glu Glu Gly Val Leu Lys Pro Tyr
 785 790 795 800
 Thr Arg Tyr Arg Leu Arg Gly Phe Val Gly Ser Ser Gln Gly Leu Glu
 805 810 815
 Ile Tyr Thr Ile Arg His Gln Thr Asn Arg Ile Val Lys Asn Val Pro
 820 825 830
 Asp Asp Leu Leu Pro Asp Val Ser Pro Val Asn Ser Asp Gly Ser Ile
 835 840 845
 Asn Arg Cys Ser Glu Gln Lys Tyr Val Asn Ser Arg Leu Glu Gly Glu
 850 855 860
 Asn Arg Ser Gly Asp Ala His Glu Phe Ser Leu Pro Ile Asp Ile Gly
 865 870 875 880
 Glu Leu Asp Tyr Asn Glu Asn Ala Gly Ile Trp Val Gly Phe Lys Ile
 885 890 895
 Thr Asp Pro Gln Gly Tyr Ala Thr Leu Gly Asn Leu Glu Leu Val Glu
 900 905 910
 Glu Gly Pro Leu Ser Gly Asp Ala Leu Glu Arg Leu Gln Arg Glu Glu
 915 920 925
 Gln Gln Trp Lys Ile Gln Met Thr Arg Arg Arg Glu Glu Thr Asp Arg
 930 935 940
 Arg Tyr Met Ala Ser Lys Gln Ala Val Asp Arg Leu Tyr Ala Asp Tyr
 945 950 955 960
 Gln Asp Gln Gln Leu Asn Pro Asp Val Glu Ile Thr Asp Leu Thr Ala
 965 970 975
 Ala Gln Asp Leu Ile Gln Ser Ile Pro Tyr Val Tyr Asn Glu Met Phe
 980 985 990
 Pro Glu Ile Pro Gly Met Asn Tyr Thr Lys Phe Thr Glu Leu Thr Asp
 995 1000 1005
 Arg Leu Gln Gln Ala Trp Asn Leu Tyr Asp Gln Arg Asn Ala Ile Pro
 1010 1015 1020
 Asn Gly Asp Phe Arg Asn Gly Leu Ser Asn Trp Asn Ala Thr Pro Gly
 1025 1030 1035 1040
 Val Glu Val Gln Gln Ile Asn His Thr Ser Val Leu Val Ile Pro Asn
 1045 1050 1055
 Trp Asp Glu In Val Ser Gln Gln Ph Thr Val Gln Pro Asn Gln Arg
 1060 1065 1070

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Tyr Val Leu Arg Val Thr Ala Arg Lys Glu Gly Val Gly Asn Gly Tyr
 1075 1080 1085
 Val Ser Ile Arg Asp Gly Gly Asn Gln Ser Glu Thr Leu Thr Phe Ser
 1090 1095 1100
 Ala Ser Asp Tyr Asp Thr Asn Gly Val Tyr Asn Asp Gln Thr Gly Tyr
 1105 1110 1115 1120
 Ile Thr Lys Thr Val Thr Phe Ile Pro Tyr Thr Asp Gln Met Trp Ile
 1125 1130 1135
 Glu Ile Ser Glu Thr Glu Gly Thr Phe Tyr Ile Glu Ser Val Glu Leu
 1140 1145 1150
 Ile Val Asp Val Glu
 1155

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1953 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Bacillus thuringiensis*
- (B) STRAIN: tolworthi
- (C) INDIVIDUAL ISOLATE: 43F

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli XL1-Blue (pM1,98-4), NRRL B-18291

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1953

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG AAT CCA AAC AAT CGA AGT GAA TAT GAT ACG ATA AAG GTT ACA CCT	48
Met Asn Pro Asn Asn Arg Ser Glu Tyr Asp Thr Ile Lys Val Thr Pro	
1 5 10 15	
AAC AGT GAA TTG CCA ACT AAC CAT AAT CAA TAT CCT TTA GCT GAC AAT	96
Asn Ser Glu Leu Pro Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn	
20 25 30	
CCA AAT TCG ACA CTA GAA GAA TTA AAT TAT AAA GAA TTT TTA AGA ATG	144
Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met	
35 40 45	
ACT GCA GAC AAT TCT ACG GAA GTG CTA GAC AGC TCT ACA GTA AAA GAT	192
Thr Ala Asp Asn Ser Thr Glu Val Leu Asp Ser Ser Thr Val Lys Asp	
50 55 60	
GCA GTT GGG ACA GGA ATT TCT GTT GTA GGA CAG ATT TTA GGT GTT GTA	240
Ala Val Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val Val	
65 70 75 80	
GGG GTT CCA TTT GCT GGG GCG CTC ACT TCA TTT TAT CAA TCA TTT CTT	288
Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr Gln Ser Phe Leu	
85 90 95	
AAC GCT ATA TGG CCA AGT GAT GCT GAC CCA TGG AAG GCT TTT ATG GCA	336
Asn Ala Ile Trp Pro Ser Asp Ala Asp Pro Trp Lys Ala Phe Met Ala	
100 105 110	
CAA GTG GAA GTA CTG ATA GAT AAG AAA ATA GAG GAG TAT GCT AAA AGT	384
Gln Val Glu Val Leu Ile Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser	
115 120 125	
AAA GCT CTT GCA GAG TTA CAG GGT CTT CAA AAT AAT TTT GAA GAT TAT	432
Lys Ala Leu Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr	
130 135 140	

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GTA Val 145	AAT Asn	GCG Ala	TTG Leu	GAT Asp	TCC Ser 150	TGG Trp	AAG Lys	AAA Lys	GCG Ala 155	CCT Pro	GTA Val	AAT Asn	TTA Leu	CGA Arg	AGT Ser 160	480
CGA Arg	AGA Arg	AGC Ser	CAA Gln	GAT Asp 165	CGA Arg	ATA Ile	AGA Arg	GAA Glu 170	CTT Leu	TTT Phe	TCT Ser	CAA Gln	GCA Ala	GAA Glu 175	AGC Ser 175	528
CAT His	TTT Phe	CGT Arg	AAT Asn 180	TCC Ser	ATG Met	CCG Pro	TCA Ser	TTT Phe 185	GCG Ala	GTT Val	TCC Ser	AAA Lys	TTT Phe 190	GAA Glu	GTT Val	576
CTG Leu	TTT Phe	CTA Leu 195	CCA Pro	ACA Thr	TAT Tyr	GCA Ala 200	CAA Gln	GCT Ala	GCA Ala	AAT Asn	ACA Thr	CAT His 205	TTA Leu	TTG Leu	CTA Leu	624
TTA Leu 210	AAA Lys	GAT Asp	GCT Ala	CAA Gln	GTT Val	TTT Phe 215	GGA Gly	GAA Glu	GAA Glu	TGG Trp	GGA Gly 220	TAT Tyr	TCT Ser	TCA Ser	GAA Glu	672
GAT Asp 225	ATT Ile	GCT Ala	GAA Glu	TTT Phe	TAT Tyr 230	CAA Gln	AGA Arg	CAA Gln	TTA Leu	AAA Lys 235	CTT Leu	ACG Thr	CAA Gln	CAA Gln	TAC Tyr 240	720
ACT Thr	GAC Asp	CAT His	TGT Cys	GTC Val 245	AAT Asn	TGG Trp	TAT Tyr	AAT Asn	GTT Val 250	GGA Gly	TTA Leu	AAT Asn	AGT Ser	TTA Leu	AGA Arg	768
GGT Gly	TCA Ser	ACT Thr	TAT Tyr 260	GAT Asp	GCA Ala	TGG Trp	GTC Val	AAA Lys 265	TTT Phe	AAC Asn	CGT Arg	TTT Phe	CGC Arg	AGA Arg	GAA Glu	816
ATG Met	ACA Thr	TTA Leu 275	ACT Thr	GTA Val	TTA Leu	GAT Asp	CTA Leu	ATT Ile	GTA Val	TTA Leu	TTC Phe	CCA Pro 285	TTT Phe	TAT Tyr	GAT Asp	864
GTT Val	CGG Arg 290	TTA Leu	TAC Tyr	TCA Ser	AAA Lys	GGA Gly 295	GTT Val	AAA Lys	ACA Thr	GAA Glu	CTA Leu 300	ACA Thr	AGA Arg	GAC Asp	ATT Ile	912
TTT Phe 305	ACA Thr	GAT Asp	CCA Pro	ATT Ile	TTT Phe 310	ACA Thr	CTC Leu	AAT Asn	GCT Ala	CTT Leu 315	CAA Gln	GAG Glu	TAT Tyr	GGA Gly	CCA Pro 320	960
ACT Thr	TTT Phe	TCG Ser	AGT Ser	ATA Ile 325	GAA Glu	AAC Asn	TCT Ser	ATT Ile	CGA Arg 330	AAA Lys	CCT Pro	CAT His	TTA Leu	TTT Phe 335	GAT Asp	1008
TAT Tyr	TTG Leu	CGT Arg	GGG Gly 340	ATT Ile	GAA Glu	TTT Phe	CAT His	ACG Thr 345	CGT Arg	CTT Leu	CGA Arg	CCT Pro	GGT Gly 350	TAC Tyr	TCT Ser	1056
GGG Gly	AAA Lys	GAT Asp 355	TCT Ser	TTC Phe	AAT Asn	TAT Tyr	TGG Trp 360	TCT Ser	GTT Gly	AAT Asn	TAT Tyr	GTA Val 365	GAA Glu	ACT Thr	AGA Arg	1104
CCT Pro	AGT Ser 370	ATA Ile	GGA Gly	TCT Ser	AAT Asn	GAT Asp 375	ACA Thr	ATC Ile	ACT Thr	TCC Ser	CCA Pro 380	TTT Phe	TAT Tyr	GGA Gly	GAT Asp	1152
AAA Lys 385	TCT Ser	ATT Ile	GAA Glu	CCT Pro	ATA Ile 390	CAA Gln	AAG Lys	CTA Leu	AGC Ser	TTT Phe 395	GAT Asp	GGA Gly	CAA Gln	AAA Lys	GTT Val 400	1200
TAT Tyr	CGA Arg	ACT Thr	ATA Ile	GCT Ala 405	AAT Asn	ACA Thr	GAC Asp	ATA Ile	GCG Ala 410	GCT Ala	TTT Phe	CCG Pro	GAT Asp	GGC Gly 415	AAG Lys	1248
ATA Ile	TAT Tyr	TTT Phe	GGT Gly 420	GTT Val	ACG Thr	AAA Lys	GTT Val	GAT Asp 425	TTT Phe	AGT Ser	CAA Gln	TAT Tyr	GAT Asp 430	GAT Asp	CAA Gln	1296
AAA Lys	AAT Asn	GAA Glu 435	ACT Thr	AGT Ser	ACA Thr	CAA Gln	ACA Thr 440	TAT Tyr	GAT Asp	TCA Ser	AAA Lys	AGA Arg	TAC Tyr	AAT Asn	GGC Gly	1344
TAT Tyr	TTA Leu 450	GGT Gly	GCA Ala	CAG Gln	GAT Asp	TCT Ser 455	ATC Ile	GAC Asp	CAA Gln	TTA Leu	CCA Pro 460	CCA Pro	GAA Glu	ACA Thr	ACA Thr	1392
GAT Asp 465	GAA Glu	CCA Pro	CTT Leu	GAA Glu	AAA Lys 470	GCA Ala	TAT Tyr	AGT Ser	CAT His	CAG Gln 475	CTT Leu	AAT Asn	TAC Tyr	GCA Ala	GAA Glu 480	1440

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TGT Cys	TTC Phe	TTA Leu	ATG Met	CAG Gln 485	GAC Asp	CGT Arg	CGT Arg	GGA Gly	ACA Thr 490	ATT Ile	CCA Pro	TTT Phe	TTT Phe	ACT Thr 495	TGG Trp	1488
ACA Thr	CAT His	AGA Arg	AGT Ser 500	GTA Val	GAC Asp	TTT Phe	TTT Phe	AAT Asn 505	ACA Thr	ATT Ile	GAT Asp	GCT Ala	GAA Glu 510	AAA Lys	ATT Ile	1536
ACT Thr	CAA Gln	CTT Leu 515	CCA Pro	GTA Val	GTG Val	AAA Lys	GCA Ala 520	TAT Tyr	GCC Ala	TTG Leu	TCT Ser	TCA Ser 525	GGC Gly	GCT Ala	TCC Ser	1584
ATT Ile 530	ATT Ile	GAA Glu	GGT Gly	CCA Pro	GGA Gly	TTC Phe 535	ACA Thr	GGA Gly	GGA Gly	AAT Asn 540	TTA Leu	CTA Leu	TTC Phe	CTA Leu	AAA Lys	1632
GAA Glu 545	TCT Ser	AGT Ser	AAT Asn	TCA Ser 550	ATT Ile	GCT Ala	AAA Lys	TTT Phe	AAA Lys	GTT Val 555	ACC Thr	TTA Leu	AAT Asn	TCA Ser	GCA Ala 560	1680
GCC Ala	TTG Leu	TTA Leu	CAA Gln 565	CGA Arg	TAT Tyr	CGC Arg	GTA Val	AGA Arg 570	ATA Ile	CGC Arg	TAT Tyr	GCT Ala	TCA Ser	ACC Thr 575	ACT Thr	1728
AAC Asn	CTA Leu	CGA Arg 580	CTT Leu	TTC Phe	GTG Val	CAA Gln	AAT Asn 585	TCA Ser	AAC Asn	AAT Asn	GAT Asp	TTT Phe 590	CTT Leu	GTC Val	ATC Ile	1776
TAC Tyr	ATT Ile	AAT Asn 595	AAA Lys	ACT Thr	ATG Met	AAT Asn	ATA Ile 600	GAT Asp	GGT Gly	GAT Asp	TTA Leu	ACA Thr 605	TAT Tyr	CAA Gln	ACA Thr	1824
TTT Phe 610	GAT Asp	TTC Phe	GCA Ala	ACT Thr	AGT Ser	AAT Asn 615	TCT Ser	AAT Asn	ATG Met	GGA Gly	TTC Phe 620	TCT Ser	GGT Gly	GAT Asp	ACA Thr	1872
AAT Asn 625	GAC Asp	TTT Phe	ATA Ile	ATA Ile	GGA Gly 630	GCA Ala	GAA Glu	TCT Ser	TTC Phe	GTT Val 635	TCT Ser	AAT Asn	GAA Glu	AAA Lys	ATC Ile 640	1920
TAT Tyr	ATA Ile	GAT Asp	AAG Lys	ATA Ile	GAA Glu 645	TTT Phe	ATC Ile	CCA Pro	GTA Val 650	CAA Gln						1953

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 651 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Bacillus thuringiensis*
 (B) STRAIN: tolworthi
 (C) INDIVIDUAL ISOLATE: 43F

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli XL1-Blue (pM1,98-4), NRRL B-18291

(ix) FEATURE:

- (A) NAME/KEY: Protein
 (B) LOCATION: 1..651

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asn Pro Asn Asn Arg Ser Glu Tyr Asp Thr Ile Lys Val Thr Pro
 1 5 10 15
 Asn Ser Glu Leu Pro Thr Asn His Asn Gln Tyr Pro Leu Ala Asp Asn
 20 25 30
 Pro Asn Ser Thr Leu Glu Glu Leu Asn Tyr Lys Glu Phe Leu Arg Met
 35 40 45

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Thr 50 Ala Asp Asn Ser Thr 55 Gln Val Leu Asp Ser 60 Thr Val Lys Asp
 Ala 65 Val Gly Thr Gly 70 Ile Ser Val Val Gly 75 Gln Ile Leu Gly Val 80 Val
 Gly Val Pro Phe 85 Ala Gly Ala Leu Thr 90 Ser Phe Tyr Gln Ser 95 Phe Leu
 Asn Ala Ile Trp 100 Pro Ser Asp Ala 105 Asp Pro Trp Lys Ala Phe Met Ala
 Gln Val Glu 115 Val Leu Ile Asp Lys 120 Lys Ile Glu Glu Tyr 125 Ala Lys Ser
 Lys Ala 130 Leu Ala Glu Leu Gln 135 Gly Leu Gln Asn 140 Asn Phe Glu Asp Tyr
 Val 145 Asn Ala Leu Asp Ser 150 Trp Lys Lys Ala Pro 155 Val Asn Leu Arg Ser 160
 Arg Arg Ser Gln 165 Asp Arg Ile Arg Gln Leu Phe Ser Gln Ala Glu Ser 175
 His Phe Arg Asn 180 Ser Met Pro Ser Phe 185 Ala Val Ser Lys Phe Glu Val 190
 Leu Phe Leu 195 Pro Thr Tyr Ala 200 Gln Ala Ala Asn Thr His 205 Leu Leu Leu
 Leu Lys 210 Asp Ala Gln Val Phe 215 Gly Glu Glu Trp Gly Tyr Ser Ser Glu 220
 Asp 225 Ile Ala Gln Phe Tyr 230 Gln Arg Gln Leu Lys 235 Leu Thr Gln Gln Tyr 240
 Thr Asp His Cys Val 245 Asn Trp Tyr Asn Val 250 Gly Leu Asn Ser Leu Arg 255
 Gly Ser Thr Tyr 260 Asp Ala Trp Val Lys 265 Phe Asn Arg Phe Arg Arg Gln 270
 Met Thr Leu 275 Thr Val Leu Asp Leu Ile Val Leu Phe Pro 285 Phe Tyr Asp
 Val Arg 290 Leu Tyr Ser Lys Gly 295 Val Lys Thr Gln Leu Thr Arg Asp Ile 300
 Phe Thr Asp Pro Ile Phe 310 Thr Leu Asn Ala Leu 315 Gln Glu Tyr Gly Pro 320
 Thr Phe Ser Ser Ile 325 Glu Asn Ser Ile Arg 330 Lys Pro His Leu Phe Asp 335
 Tyr Leu Arg Gly 340 Ile Glu Phe His Thr 345 Arg Leu Arg Pro Gly 350 Tyr Ser
 Gly Lys Asp 355 Ser Phe Asn Tyr Trp 360 Ser Gly Asn Tyr Val Glu Thr Arg 365
 Pro Ser Ile Gly Ser Asn Asp 375 Thr Ile Thr Ser Pro 380 Phe Tyr Gly Asp 385
 Lys Ser Ile Glu Pro Ile Gln Lys Leu Ser Phe 395 Asp Gly Gln Lys Val 400
 Tyr Arg Thr Ile Ala 405 Asn Thr Asp Ile Ala 410 Ala Phe Pro Asp Gly Lys 415
 Ile Tyr Phe Gly 420 Val Thr Lys Val Asp 425 Phe Ser Gln Tyr Asp Asp Gln 430
 Lys Asn Glu 435 Thr Ser Thr Gln Thr 440 Tyr Asp Ser Lys Arg 445 Tyr Asn Gly
 Tyr Leu Gly Ala Gln Asp Ser 455 Ile Asp Gln Leu Pro 460 Pro Glu Thr Thr 465
 Asp Glu Pro Leu Glu Lys 470 Ala Tyr Ser His Gln 475 Leu Asn Tyr Ala Gln 480
 Cys Phe Leu Met Gln 485 Asp Arg Arg ly Thr 490 Ile Pro Phe Phe Thr 495 Trp

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Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp Ala Glu Lys Ile
 500 505 510
 Thr Gln Leu Pro Val Val Lys Ala Tyr Ala Leu Ser Ser Gly Ala Ser
 515 520 525
 Ile Ile Glu Gly Pro Gly Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys
 530 535 540
 Glu Ser Ser Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
 545 550 555 560
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr
 565 570 575
 Asn Leu Arg Leu Phe Val Gln Asn Ser Asn Asn Asp Phe Leu Val Ile
 580 585 590
 Tyr Ile Asn Lys Thr Met Asn Ile Asp Gly Asp Leu Thr Tyr Gln Thr
 595 600 605
 Phe Asp Phe Ala Thr Ser Asn Ser Asn Met Gly Phe Ser Gly Asp Thr
 610 615 620
 Asn Asp Phe Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu Lys Ile
 625 630 635 640
 Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln
 645 650

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1425 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: BACILLUS THURINGIENSIS
- (C) INDIVIDUAL ISOLATE: PS86A1

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli NM522(pMYC2320) NRRL B-18769

(ix) FEATURE:

- (A) NAME/KEY: nat_peptide
- (B) LOCATION: 1..1425

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGATTATTG ATAGTAAAC GACTTTACCT AGACATTCAC TTATTCATAC AATTAAATTA	60
AATTCTAATA AGAAATATCG TCCTGGTGAT ATGACTAATG GAAATCAATT TATTATTTC	120
AAACAAGAAT GGGCTACGAT TGGAGCATAT ATTCAGACTG GATTAGGTTT ACCAGTAAAT	180
GAACAACAAT TAAGACACA TGTTAATTTA AGTCAGGATA TATCAATACC TAGTGATTTT	240
TCTCAATTAT ATGATGTTTA TTGTTCTGAT AAAACTTCAG CAGATGGTG GAATAAAAT	300
TTATATCCTT TAATTATTAA ATCTGCTAAT GATATTGCTT CATATGGTTT TAAAGTTGCT	360
GGTGATCCTT CTATTAAGAA AGATGGATAT TTTAAAAAAT TGCAAGATGA ATTAGATAAT	420
ATTGTTGATA ATAATCCGA TGATGATGCA ATAGCTAAG CTATTAAAGA TTTTAAAGCG	480
CGATGTGGTA TTTTAATTAA AGAAGCTAAA CAATATGAAG AAGCTGCAA AAATATTGTA	540
ACATCTTTAG ATCAATTTT ACATGGTGAT CAGAAAAAAT TAGAAGGTGT TATCAATATT	600
CAAAAACGTT TAAAAGAAGT TCAAACAGCT CTTAATCAAG CCCATGGGGA AAGTAGTCCA	660
GCTCATAAAG AGTTATTAGA AAAAGTAAAA AATTTAAAA CAACATTAGA AAGGACTATT	720
AAAGCTGAAC AAGATTTAGA GAAAAAGTA GAATATAGTT TTCTATTAGG ACCATTGTTA	780

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CGATTGTGTTG TTTATGAAT TCTTGAAAT ACTGCTGTT AGCATATAAA AAATCAAATT 840
GATGAGATAA AGAACAATT AGATTCTGCT CAGCATGATT TGGATAGAGA TGTAAAATT 900
ATAGGAATGT TAAATAGTAT TAATACAGAT ATTGATAATT TATATAGTCA AGGACAAGAA 960
GCAATTAAG TTTCCAAA GTTACAAGGT ATTGGGCTA CTATTGGAGC TCAAATAGAA 1020
AATCTTAGAA CAACGTCGTT ACAAGAAGTT CAAGATTCTG ATGATGCTGA TGGAGATACAA 1080
ATTGAACCTG AGGACGCTTC TGATGCTGG TTAGTTGTTG CTCAGAAGC TCGTGATTTT 1140
ACACTAAATG CTTATTCAAC TAATAGTAGA CAAATTTAC CGATTATGT TATATCAGAT 1200
TCATGTAATT GTTCAACAAC AAATATGACA TCAATCAAT ACAGTAATCC AACACAAAT 1260
ATGACATCAA ATCAATATAT GATTTACAT GAATATACAA GTTTACCAA TAATTTTATG 1320
TTATCAAGAA ATAGTAATTT AGAATATAAA TGTCCTGAAA ATAATTTTAT GATATATTGG 1380
TATAATAATT CGGATTGGTA TAATAATTCG GATTGGTATA ATAAT 1425

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(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 475 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: BACILLUS THURINGIENSIS
 (C) INDIVIDUAL ISOLATE: PS86A1
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: E. coli NM522(pMYC2320) NRRL B-18769
- (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..475
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
- ```

Met Ile Ile Asp Ser Lys Thr Thr Leu Pro Arg His Ser Leu Ile His
1 5 10
Thr Ile Lys Leu Asn Ser Asn Lys Lys Tyr Gly Pro Gly Asp Met Thr
20 25 30
Asn Gly Asn Gln Phe Ile Ile Ser Lys Gln Glu Trp Ala Thr Ile Gly
35 40 45
Ala Tyr Ile Gln Thr Gly Leu Gly Leu Pro Val Asn Glu Gln Gln Leu
50 55 60
Arg Thr His Val Asn Leu Ser Gln Asp Ile Ser Ile Pro Ser Asp Phe
65 70 75 80
Ser Gln Leu Tyr Asp Val Tyr Cys Ser Asp Lys Thr Ser Ala Glu Trp
85 90 95
Trp Asn Lys Asn Leu Tyr Pro Leu Ile Ile Lys Ser Ala Asn Asp Ile
100 105 110
Ala Ser Tyr Gly Phe Lys Val Ala Gly Asp Pro Ser Ile Lys Lys Asp
115 120 125
Gly Tyr Phe Lys Lys Leu Gln Asp Glu Leu Asp Asn Ile Val Asp Asn
130 135 140
Asn Ser Asp Asp Asp Ala Ile Ala Lys Ala Ile Lys Asp Phe Lys Ala
145 150 155 160
Arg Cys Gly Ile Leu Ile Lys Glu Ala Lys Gln Tyr Glu Glu Ala Ala
165 170 175
Lys Asn Ile Val Thr Ser Leu Asp Gln Phe Leu His Gly Asp Gln Lys

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| 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Leu | Glu | Gly | Val | Ile | Asn | Ile | Gln | Lys | Arg | Leu | Lys | Glu | Val | Gln |
|     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |
| Thr | Ala | Leu | Asn | Gln | Ala | His | Gly | Gln | Ser | Ser | Pro | Ala | His | Lys | Glu |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Leu | Leu | Glu | Lys | Val | Lys | Asn | Leu | Lys | Thr | Thr | Leu | Glu | Arg | Thr | Ile |
|     | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     | 240 |
| Lys | Ala | Glu | Gln | Asp | Leu | Glu | Lys | Lys | Val | Glu | Tyr | Ser | Phe | Leu | Leu |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Gly | Pro | Leu | Leu | Gly | Phe | Val | Val | Tyr | Glu | Ile | Leu | Glu | Asn | Thr | Ala |
|     |     |     |     | 260 |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Val | Gln | His | Ile | Lys | Asn | Gln | Ile | Asp | Glu | Ile | Lys | Lys | Gln | Leu | Asp |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Ser | Ala | Gln | His | Asp | Leu | Asp | Arg | Asp | Val | Lys | Ile | Gly | Met | Leu |     |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Asn | Ser | Ile | Asn | Thr | Asp | Ile | Asp | Asn | Leu | Tyr | Ser | Gln | Gly | Gln | Glu |
|     | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     | 320 |
| Ala | Ile | Lys | Val | Phe | Gln | Lys | Leu | Gln | Gly | Ile | Trp | Ala | Thr | Ile | Gly |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Ala | Gln | Ile | Glu | Asn | Leu | Arg | Thr | Thr | Ser | Leu | Gln | Glu | Val | Gln | Asp |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |
| Ser | Asp | Asp | Ala | Asp | Glu | Ile | Gln | Ile | Glu | Leu | Glu | Asp | Ala | Ser | Asp |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| Ala | Trp | Leu | Val | Val | Ala | Gln | Glu | Ala | Arg | Asp | Phe | Thr | Leu | Asn | Ala |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |
| Tyr | Ser | Thr | Asn | Ser | Arg | Gln | Asn | Leu | Pro | Ile | Asn | Val | Ile | Ser | Asp |
|     | 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     | 400 |
| Ser | Cys | Asn | Cys | Ser | Thr | Thr | Asn | Met | Thr | Ser | Asn | Gln | Tyr | Ser | Asn |
|     |     |     | 405 |     |     |     |     |     | 410 |     |     |     | 415 |     |     |
| Pro | Thr | Thr | Asn | Met | Thr | Ser | Asn | Gln | Tyr | Met | Ile | Ser | His | Glu | Tyr |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |
| Thr | Ser | Leu | Pro | Asn | Asn | Phe | Met | Leu | Ser | Arg | Asn | Ser | Asn | Leu | Glu |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |
| Tyr | Lys | Cys | Pro | Glu | Asn | Asn | Phe | Met | Ile | Tyr | Trp | Tyr | Asn | Asn | Ser |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |
| Asp | Trp | Tyr | Asn | Asn | Ser | Asp | Trp | Tyr | Asn | Asn |     |     |     |     |     |
|     | 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     |

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 53 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Bacillus thuringiensis*
- (B) STRAIN: PS86A1

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGATTGATT CTAAACAAC ATTACCAAGA CATTCTTAA TWCATACAT WAA

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Claims

- 1           1. A method for controlling lepidopteran insect pests which comprises contacting said  
2 insect pests with an insect-controlling effective amount of a *Bacillus thuringiensis* isolate selected  
3 from the group consisting of *B.t.* PS43F, *B.t.* PS50C and *B.t.* PS86A1, and variants thereof.
- 1           2. The method, according to claim 1, wherein said *Bacillus thuringiensis* is *B.t.* PS43F.
- 1           3. The method, according to claim 1, wherein said *Bacillus thuringiensis* is *B.t.* PS50C.
- 1           4. The method, according to claim 1, wherein said *Bacillus thuringiensis* is *B.t.* PS86A1.
- 1           5. The method, according to claim 1, wherein said insect pest is the diamondback moth  
2 (*Plutella xylostella*).
- 1           6. The method, according to claim 1, which comprises applying an insecticidal  
2 composition comprising *Bacillus thuringiensis* to plants or soil.
- 1           7. The method, according to claim 6, wherein said insecticidal composition is a liquid.
- 1           8. The method, according to claim 6, wherein said insecticidal composition is in granular  
2 form.
- 1           9. The method, according to claim 6, wherein said insecticidal composition is applied  
2 when corn or soybean seed is planted.
- 1           10. The method, according to claim 1, wherein said *Bacillus thuringiensis* are treated to  
2 prolong their pesticidal activity in the environment of a target pest.
- 1           11. A method for controlling lepidopteran pests which comprises exposing said pests to  
2 a plant transformed by a gene obtainable from a *Bacillus thuringiensis* isolate selected from the  
3 group consisting of *B.t.* PS43F, *B.t.* PS50C and *B.t.* PS86A1, and variants thereof, wherein said  
4 gene encodes a toxin active against lepidopteran pests.
- 1           12. The method, according to claim 11, wherein said gene comprises the DNA of SEQ  
2 ID NO. 1 or a portion thereof which encodes a lepidopteran-active toxin.

1           13. The method, according to claim 11, wherein said gene comprises the DNA of SEQ  
2 ID NO. 3 or a portion thereof which encodes a lepidopteran-active toxin.

1           14. The method, according to claim 11, wherein said gene comprises the DNA of SEQ  
2 ID NO. 5 or a portion thereof which encodes a lepidopteran-active toxin.

1           15. A method for controlling lepidopteran insects which comprises administering to said  
2 insects or to the environment of said insects a microorganism transformed by a gene obtainable  
3 from a *Bacillus thuringiensis* isolate selected from the group consisting of *B.t.* PS43F, *B.t.* PS50C  
4 and *B.t.* PS86A1, and variants thereof, wherein said gene encodes a toxin active against  
5 lepidopteran pests.

1           16. The method, according to claim 15, wherein said gene comprises the DNA of SEQ  
2 ID NO. 1 or a portion thereof which encodes a lepidopteran-active toxin.

1           17. The method, according to claim 15, wherein said gene comprises the DNA of SEQ  
2 ID NO. 3 or a portion thereof which encodes a lepidopteran-active toxin.

1           18. The method, according to claim 15, wherein said gene comprises the DNA of SEQ  
2 ID NO. 5 or a portion thereof which encodes a lepidopteran-active toxin.

1           19. The method, according to claim 15, wherein said microorganism is a *Pseudomonas*.

1           20. The method, according to claim 15, wherein said transformed microorganism is  
2 treated to prolong its pesticidal activity in the environment of a target pest.

# INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US 92/07697**

| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>8</sup><br>According to International Patent Classification (IPC) or to both National Classification and IPC<br><b>IPC5: A 01 N 63/00, 63/02, C 12 N 15/32</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                           |                                     |                                                                                                                                                                                                                   |                                                                                                                                                                                                                           |                                                                                                                                                                                                                   |                                                                                                                                                                                                      |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|------|---|-----------------------------------------------------------------------------------------------------------------------------------------------------|------|---|----------------------------------------------------------------------------------------------------------------------------------------|------|
| <b>II. FIELDS SEARCHED</b><br><div style="text-align: right; font-size: small;">Minimum Documentation Searched<sup>7</sup></div> <table style="width: 100%; border: none;"> <tr> <td style="width: 25%; border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Classification System</div> </td> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Classification Symbols</div> </td> </tr> <tr> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">IPC5</div> </td> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">A 01 N; C 12 N</div> </td> </tr> </table> <div style="text-align: center; font-size: x-small; margin-top: 5px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched<sup>8</sup></div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                           |                                     | <div style="border: 1px solid black; padding: 2px;">Classification System</div>                                                                                                                                   | <div style="border: 1px solid black; padding: 2px;">Classification Symbols</div>                                                                                                                                          | <div style="border: 1px solid black; padding: 2px;">IPC5</div>                                                                                                                                                    | <div style="border: 1px solid black; padding: 2px;">A 01 N; C 12 N</div>                                                                                                                             |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |
| <div style="border: 1px solid black; padding: 2px;">Classification System</div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <div style="border: 1px solid black; padding: 2px;">Classification Symbols</div>                                                                                                                                          |                                     |                                                                                                                                                                                                                   |                                                                                                                                                                                                                           |                                                                                                                                                                                                                   |                                                                                                                                                                                                      |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |
| <div style="border: 1px solid black; padding: 2px;">IPC5</div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | <div style="border: 1px solid black; padding: 2px;">A 01 N; C 12 N</div>                                                                                                                                                  |                                     |                                                                                                                                                                                                                   |                                                                                                                                                                                                                           |                                                                                                                                                                                                                   |                                                                                                                                                                                                      |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; font-size: x-small;">Category *</th> <th style="width: 60%; font-size: x-small;">Citation of Document,<sup>11</sup> with indication, where appropriate, of the relevant passages<sup>12</sup></th> <th style="width: 30%; font-size: x-small;">Relevant to Claim No.<sup>13</sup></th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top;">P,X</td> <td style="vertical-align: top;"> <b>EP, A2, 0498537 (MYCOGEN CORPORATION)</b><br/>           12 August 1992,<br/>           see the whole document<br/> <div style="text-align: center;">—</div> </td> <td style="text-align: center; vertical-align: top;">1-20</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td style="vertical-align: top;"> <b>EP, A1, 0337604 (MYCOGEN CORPORATION)</b><br/>           18 October 1989,<br/>           see the claims; page 2, lines 30-37<br/> <div style="text-align: center;">—</div> </td> <td style="text-align: center; vertical-align: top;">1-20</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td style="vertical-align: top;"> <b>US, A, 4849217 (GEORGE G. SOARES ET AL.)</b><br/>           18 July 1989,<br/>           see the whole document<br/> <div style="text-align: center;">—</div> </td> <td style="text-align: center; vertical-align: top;">1-20</td> </tr> </tbody> </table> <div style="font-size: x-small; margin-top: 10px;"> <p>* Special categories of cited documents:<sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Z" document member of the same patent family</p> </div> |                                                                                                                                                                                                                           |                                     | Category *                                                                                                                                                                                                        | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>                                                                                                            | Relevant to Claim No. <sup>13</sup>                                                                                                                                                                               | P,X                                                                                                                                                                                                  | <b>EP, A2, 0498537 (MYCOGEN CORPORATION)</b><br>12 August 1992,<br>see the whole document<br><div style="text-align: center;">—</div> | 1-20 | X | <b>EP, A1, 0337604 (MYCOGEN CORPORATION)</b><br>18 October 1989,<br>see the claims; page 2, lines 30-37<br><div style="text-align: center;">—</div> | 1-20 | X | <b>US, A, 4849217 (GEORGE G. SOARES ET AL.)</b><br>18 July 1989,<br>see the whole document<br><div style="text-align: center;">—</div> | 1-20 |
| Category *                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>                                                                                                            | Relevant to Claim No. <sup>13</sup> |                                                                                                                                                                                                                   |                                                                                                                                                                                                                           |                                                                                                                                                                                                                   |                                                                                                                                                                                                      |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |
| P,X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | <b>EP, A2, 0498537 (MYCOGEN CORPORATION)</b><br>12 August 1992,<br>see the whole document<br><div style="text-align: center;">—</div>                                                                                     | 1-20                                |                                                                                                                                                                                                                   |                                                                                                                                                                                                                           |                                                                                                                                                                                                                   |                                                                                                                                                                                                      |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <b>EP, A1, 0337604 (MYCOGEN CORPORATION)</b><br>18 October 1989,<br>see the claims; page 2, lines 30-37<br><div style="text-align: center;">—</div>                                                                       | 1-20                                |                                                                                                                                                                                                                   |                                                                                                                                                                                                                           |                                                                                                                                                                                                                   |                                                                                                                                                                                                      |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <b>US, A, 4849217 (GEORGE G. SOARES ET AL.)</b><br>18 July 1989,<br>see the whole document<br><div style="text-align: center;">—</div>                                                                                    | 1-20                                |                                                                                                                                                                                                                   |                                                                                                                                                                                                                           |                                                                                                                                                                                                                   |                                                                                                                                                                                                      |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |
| <b>IV. CERTIFICATION</b> <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Date of the Actual Completion of the International Search</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px;">27th November 1992</div> </td> <td style="width: 50%; border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Date of Mailing of this International Search Report</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px; text-align: center;">315 DEC 1992</div> </td> </tr> <tr> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">International Searching Authority</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px; text-align: center;">EUROPEAN PATENT OFFICE</div> </td> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Signature of Authorized Officer</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px; text-align: center;">Gerd Wranne</div> </td> </tr> </table>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                           |                                     | <div style="border: 1px solid black; padding: 2px;">Date of the Actual Completion of the International Search</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px;">27th November 1992</div> | <div style="border: 1px solid black; padding: 2px;">Date of Mailing of this International Search Report</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px; text-align: center;">315 DEC 1992</div> | <div style="border: 1px solid black; padding: 2px;">International Searching Authority</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px; text-align: center;">EUROPEAN PATENT OFFICE</div> | <div style="border: 1px solid black; padding: 2px;">Signature of Authorized Officer</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px; text-align: center;">Gerd Wranne</div> |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |
| <div style="border: 1px solid black; padding: 2px;">Date of the Actual Completion of the International Search</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px;">27th November 1992</div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <div style="border: 1px solid black; padding: 2px;">Date of Mailing of this International Search Report</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px; text-align: center;">315 DEC 1992</div> |                                     |                                                                                                                                                                                                                   |                                                                                                                                                                                                                           |                                                                                                                                                                                                                   |                                                                                                                                                                                                      |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |
| <div style="border: 1px solid black; padding: 2px;">International Searching Authority</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px; text-align: center;">EUROPEAN PATENT OFFICE</div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <div style="border: 1px solid black; padding: 2px;">Signature of Authorized Officer</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px; text-align: center;">Gerd Wranne</div>                      |                                     |                                                                                                                                                                                                                   |                                                                                                                                                                                                                           |                                                                                                                                                                                                                   |                                                                                                                                                                                                      |                                                                                                                                       |      |   |                                                                                                                                                     |      |   |                                                                                                                                        |      |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) |                                                                                       |                      |
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